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## PREPARATION AND EVALUATION OF NEVIRAPINE-LOADED EUDRAGIT L100 NANOPARTICLES BY SOLVENT EVAPORATION TECHNIQUE

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### ABSTRACT

**Aim:** The aim of the present study is to prepare and characterize nanoparticles containing Nevirapine using Eudragit L 100 as the polymer. **Methods:** The Nevirapine loaded nanoparticles were prepared by Solvent evaporation method. Nanoparticles of different core: coat ratio were prepared and characterize for process yield, loading efficiency, particle size, zeta potential, *in vitro* drug release, kinetic study and stability study. **Results:** The organized nanoparticles be spherical in shape. The infrared spectra and differential scanning calorimetry thermographs showed stable character of Nevirapine in the drug-loaded nanoparticles and revealed the absence of drug polymer interactions. The formulation FE-3 registered has best formulation. The Eudragit L100 nanoparticles have a particle diameter ranging approximately 642.4 nm and a zeta potential -9.26 mV. The *in vitro* release behavior from all the drug loaded batches were found to follow first order and provided sustained release over a period of 12 h. No appreciable difference was observed in the extent of degradation of product during 90 days in which nanoparticles were stored on different temperatures. **Conclusion:** The best-fit release kinetics be achieved through First order followed by Higuchi plot. The release of Nevirapine was influenced by the drug to particle size and polymer ratio and was Initiate to be diffusion controlled. According to the data obtained, this Eudragit L100-based nanoparticles opens new and interesting perspectives as drug carriers for treating the AIDS.

### KEYWORDS

Nanoparticle, Eudragit L 100, Nevirapine and Solvent evaporation method.

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### INTRODUCTON

Acquired Immunodeficiency Syndrome (AIDS) was Initial reported in 1981 in San Francisco<sup>1</sup>. The human immunodeficiency virus (HIV) is a retrovirus which infects the cell of immune system then destroying these cells and damages the immune system's capability to fight with the invaders<sup>2</sup>. Acquired immunodeficiency syndrome (AIDS) is a serious disease afflicting many populations of the world. Many classes of the drugs

are used in the healing of AIDS. Of these, nonnucleoside reverse transcriptase inhibitors (NNRTIs) are a specific class of anti AIDS drugs<sup>3</sup>. Some of the NNRTIs use is limited due to low bioavailability resulting from dissolution rate-limited bioavailability. Their bioavailability can be improved by formulating as Nanoparticle.

In this study we chose nevirapine, a BCS class II NNRTI with undesirable solubility and dissolution kinetics from the dosage form. Its solubility in neutral pH is about 0.1 mg/ml. even though the drug appears to be well absorbed orally, at higher doses, its bioavailability is low and variable<sup>4</sup>. On occasions it is administered as 200 mg twice daily. Increasing the dose to 400 mg to provide once daily dosing resulted in variable bioavailability profiles. This has been attributed to its limited solubility and dissolution rate-limited absorption. Developing novel Nanoparticle formulations might help reduce this variability in bioavailability.

Nevirapine is available as oral tablets as well as oral paediatric suspension. These conventional formulations of nevirapine were approved during 1996-98<sup>5</sup>. Later on, extended release formulations were also approved for clinical use. Clinical trials conducted during the beginning of this century proved so as to better than indinavir, nelfinavir and efavirenz. Because of its high potency, no food effect, low pill burden and low cost, combination of nevirapine with other anti AIDS drugs has been used as the first line therapy in developing countries. The innovator of this product is Boehringer Ingelheim Pharmaceuticals, USA and patents with conventional dosage form have already expired and at present few generic products are available in the USA and other regulated markets. Challenges still remain in the area of research for developing products of nevirapine to further enhance its solubility and reduce fluctuations in bioavailability<sup>6</sup>.

Special drug carrier systems such as nanoparticles hold the promise of overcoming these pharmacokinetic obstacles to bring about successful therapy. Nanoparticles are stable, solid colloidal particles consisting of macromolecular material and ranging in size from 10 to 10000 nm. Drugs can be adsorbed on the particle surface or can be entrapped or dissolved in the particle matrix. Various

techniques for the preparation of nanoparticles have been employed for a large number of anti-HIV drugs. Subsequent intravenous administration, nanoparticles are known to accumulate in the tissues of mononuclear phagocytic system (MPS) because of phagocytosis by monocyte/macrophages (Mo/Mac). This results in a particular enrichment of nanoparticles in macrophage-containing organs like the liver and spleen. Infection of these cells with HIV does not abrogate their phagocytic activity *in vitro* or *in vivo*. Therefore nanoparticles represent an interesting carrier system for the specific transport of ARV agents. With this drug targeting technology, substances whose improvement has been halted because of their adverse pharmacokinetic properties could potentially be made available for the treatment of HIV-related diseases. Due to greater stability and easier manufacturing, polymeric nanocarriers offer advantages over further nanocarrier systems such as liposomes and niosomes. This article gives a comprehensive review on the recent developments of polymeric nanocarrier system related to anti-HIV drugs and things to see the fields of further investigation<sup>7</sup>.

Eudragit 1 100 is a white, free flowing powder with at least 95% of dry polymers. It is an anionic copolymerization product of methyl methacrylate and methacrylic acid. It is soluble at pH.6. The ratio of free carboxyl groups to the ester is about 1:1 in Eudragit 1 100. It is readily soluble in neutral to weakly alkaline conditions (pH6-7) and from salts with alkalis, thus affording film coats which are resistant to gastric media, but so lube in intestinal fluid. It is freely soluble in alcohol, acetone, and sodium hydroxide. It is unsolvable in dichloromethane, ethyl acetate, petroleum ether and water. Nevirapine and Eudragit 1 100 were selected as core and coat material for the formulation of nanoparticles to achieve controlled drug release<sup>8</sup>.

Hence, the objective of the work was to formulate Eudragit 1100 nanoparticles containing Nevirapine by Solvent evaporation method, evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and *in vitro* release characteristics.

## MATERIAL AND METHODS

Nevirapine used was a gift sample from Strides Arcolab Pvt. Ltd. Bangalore and Eudragit 1100 from Rhom Pharm, gbbh, Germany. PVA were obtained from SD fine chemical Ltd, Mumbai, India. All other chemicals used were of analytical grade.

### Preparation of nanoparticles<sup>9</sup>

Nevirapine nanoparticle were prepared by solvent evaporation method. Drug and polymer were dissolved in 10 ml of methanol and 10 ml of dichloromethane and this solution was added to 10 ml of an aqueous PVA (2% w/v) solution. The resultant mixture was sonicated for 5 min to obtain an o/w emulsion was immediately added drop-wise to 125 ml of an aqueous PVA (2% w/v) solution. The contents were stirred at system allowing the formation of a turbid particulate suspension. The nanoparticles were separated by centrifugation 1000g for 30 min.

### Characterization of prepared nanoparticles

#### Fourier transform infra-red spectroscopy (FT-IR) analysis

The IR spectra of the samples be recorded on an FTIR spectrophotometer (Perkin Elmer 1600 series) with KBr pellet (12 mm disc), compressed in a hydraulic press at 10 tons for 30 seconds.

#### Practical yield<sup>10</sup>

The nanoparticles were collected and weighed to determine practical yield (PY) from the following equation 1.

$$PY (\%) = \frac{\text{Nanoparticles weight}}{\text{Theoretical mass}} \times 100\% \quad (1)$$

The individual values were determined for three replicates and their mean values are reported.

#### Drug content<sup>11</sup>

The drug content in each formulation was determined by weighing nanoparticles equivalent to 30mg of Nevirapine and dissolving in 100 ml of 7.4 pH phosphate buffer, followed by stirring. The solution was filtered through a 0.45 $\mu$  membrane filter, diluted suitably and the absorbance of resultant solution was measured spectro photometrically at 282 nm using 7.4 pH phosphate buffer as blank. The drug content of the prepared Nanoparticles was determined by the formula:

$$\text{Drug content (\%)} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles}} \times 100$$

#### Entrapment efficiency (EE %)<sup>12</sup>

The entrapment efficiency is also known as Association Efficiency. The nanoparticles of drug loaded were centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer (Das *et al.*, 2005).

Efficiency (DEE) was calculated as follows:

$$DEE \% = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

#### Scanning Electron Microscopy<sup>13</sup>

The shape and surface topography of nanoparticles were examined by means of Scanning Electron Microscopy (SEM) (JSM-T20. Tokyo, Japan). An appropriate sample of polymeric nanoparticles was mounted on metal stubs, using double- sided adhesive tapes. Samples were gold coated and observed for morphology, at acceleration voltage of 15KV.

#### Particle size distribution<sup>14</sup>

The size distributions along the volume mean diameters of the suspending particles were measured by dynamic scattering particle size analyser (Nanotrak Particle Analyzer 150, Microtrac Inc., PA, USA) (Alexis *et al.*, 2008).

#### In vitro release studies<sup>15</sup>

The release of drug was determined by using the treated egg membrane mounted on the one end of open tube, containing drug equivalent to 10 mg of formulation. The dialysis tube was suspended in 250 ml beaker, containing 200 ml PBS (pH 7.4). The solution was stirred at 200 rpm with the help of magnetic stirrer at 37 $\pm$ 0.5 °C. Perfect sink conditions were maintained in the drug release testing. The samples were withdrawn at proper time interval at (1, 2, 3, 4, 6, 8, 12). The dissolution medium was replaced with same amount of fresh PBS (pH 7, 4) solution to maintain the volume 200 ml throughout the experiment. The drug content in the withdrawn samples (5ml) were estimated at 282 nm and cumulative % of drug released was calculated and plotted against time (t).

#### Kinetic modeling<sup>16</sup>

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with different kinetic equation like zero order (cumulative % release v/s time), first order (log % drug remaining v/s time),

Higuchi's model (cumulative % drug release v/s square root of time), Peppas plot (log of cumulative % drug release v/s log time). R2 and 'n' values were calculated for the linear curve obtained by regression analysis of the above plots (Table No.2).

**Stability study**

The stability study was carried out using the batch FE-4. Formulation FE-4 was divided into 3 sets of samples and stored at 5±3°C in refrigerator, room temperature and 45 ± 2°C, 75% RH in humidity control ovens. After 90 days drug content of all samples were determined through the method as in drug content (Figure No.7). *In vitro* release study of formulation FE-4 was also carried out after 90 days of storage (Table No.3 and Figure No.5).

**RESULTS AND DISCUSSION**

Nanoparticles prepared by Solvent evaporation technique were found to be discrete and through SEM analysis. The drug entrapment efficiency of nanoparticles containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 74.95%, 77.86%, 79.48%, 91.73% and 81.29%. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The interaction study in between the drug and polymer was evaluated using FT-IR spectrophotometer. There was no significant difference in the IR spectra of drug loaded nanoparticles. Differential scanning calorimetry study thermo gram of pure nevirapine showed a sharp endothermic peak at 251.72°C. The thermo grams of formulations FE-3 of Figure No.2, showed the identical endothermic peak at the similar temperature. This further confirmed that there is no drug to polymer interaction. This indicates that they are stable.

Cumulative percentage drug released for FE-1, FE-2, FE-3, FE-4 and FE-5 after 12 h were found to be 86.36%, 82.53%, 78.26%, 66.81% and 70.63% respectively. Zeta potential for FE-3 was found to be -9.26mV and it shows good stability. It was apparent that *in vitro* release of Nevirapine showed a very rapid initial burst and then followed by a very slow drug release. An initial, fast release suggests that some drug was contained on the surface of the nanoparticles. In order to explain the release kinetics of all five formulations the corresponding dissolution data were fitted in different kinetic dissolution models like zero order, first order, and Higuchi respectively.

As indicated by higher R2 values, the drug release from all formulations follows first order release and Higuchi model. Because it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, Non-fickian diffusion or zero order. 'n' value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be greater than 0.50. This indicates that the release approximates Non-fickian diffusion mechanism.

**Table No.1: Formulation and physicochemical characterization of Nevirapine nanoparticles**

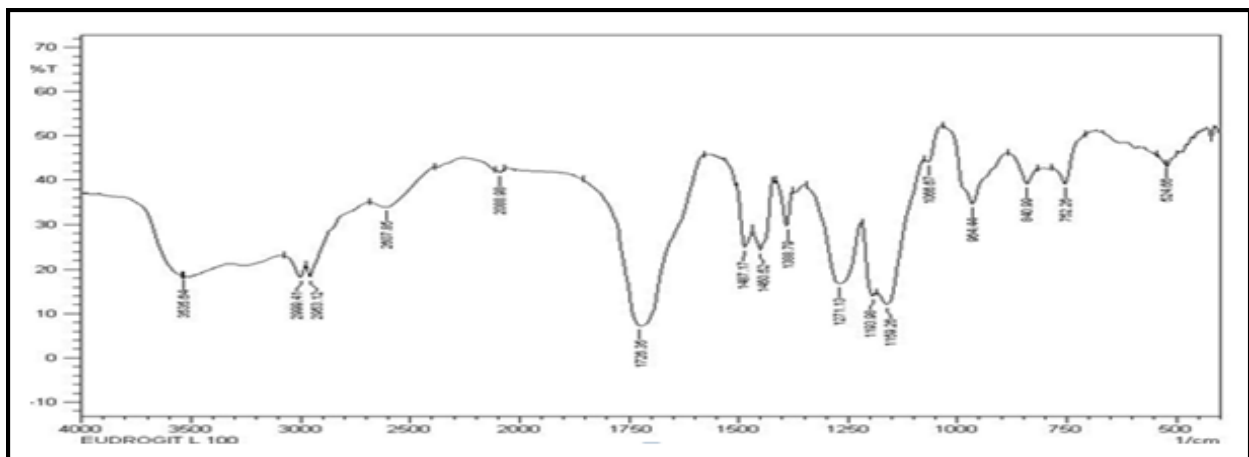
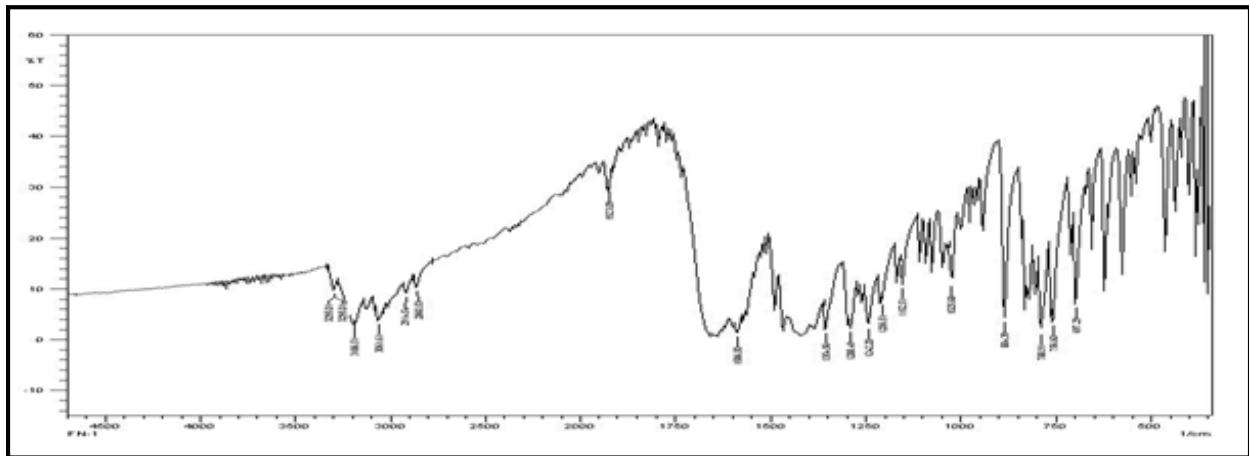
S.No	Batch code	Drug: Polymer ratio	% Drug entrapment efficiency
1	FE1	1:1	74.95
2	FE2	1:2	77.86
3	FE3	1:3	79.48
4	FE4	1:4	91.73
5	FE5	1:5	81.29

**Table No.2: Correlation coefficients according to different kinetic equations**

S.No	Formulation code	% cumulative drug release	Zero order	First order	Higuchi	Peppas	'n' values
1	FE1	86.34	0.8612	0.9743	0.9495	0.4573	1.0852
2	FE2	82.51	0.9124	0.9852	0.9832	0.5084	1.1183
3	FE3	78.26	0.8910	0.9664	0.9673	0.4758	1.0766
4	FE4	66.82	0.9413	0.9846	0.9920	0.5137	1.0615
5	FE5	70.64	0.9097	0.9691	0.9862	0.5726	1.1387

**Table No.3: Stability studies - *In vitro* release study of a selected formulation FE-3 after three months storage at 5±3 °C, Room temperature, 45±2 °C/75%RH**

S.No	Time in hrs	% cumulative Drug Release		
		5°C±3°C	30°C±2°C/65% ±5% RH	40°C±2°C/75% ±5% RH
1	0	0	0	0
2	1	23.36	21.50	18.97
3	2	30.60	29.34	26.89
4	3	38.79	37.73	35.06
5	4	42.66	41.58	38.79
s6	6	50.34	48.87	46.08
7	8	58.26	56.45	54.12
8	12	66.52	64.56	61.92



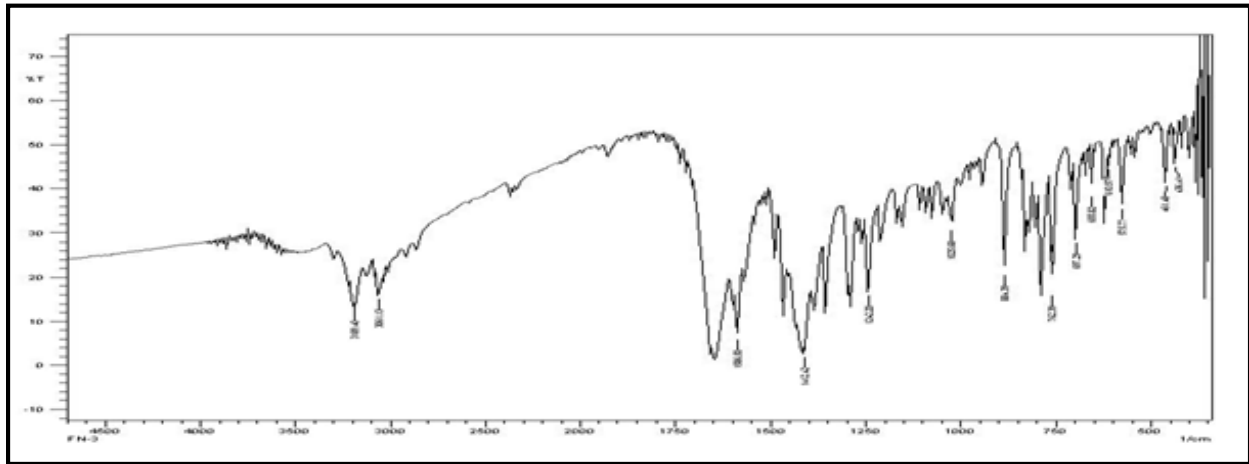
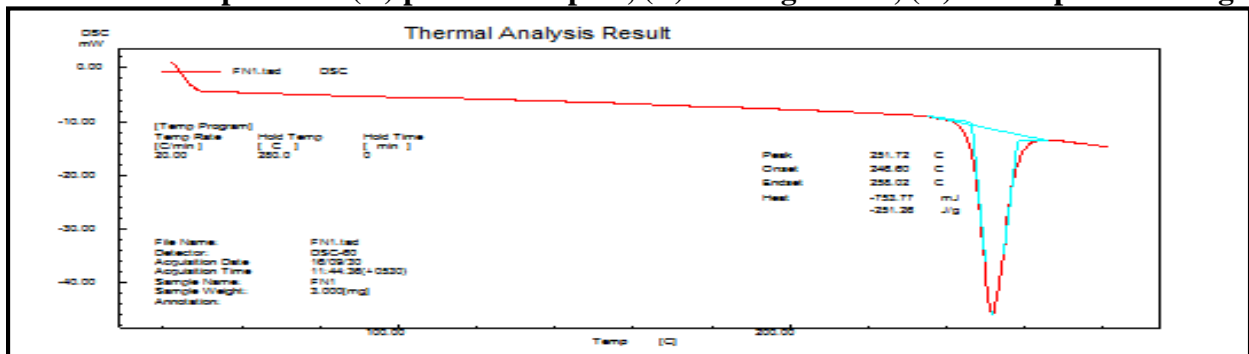


Figure No.1: FT-IR spectra of (A) pure Nevirapine, (B) Eudragit L100, (C) Nevirapine+Eudragit L 100



Drug+Eudragit L 100

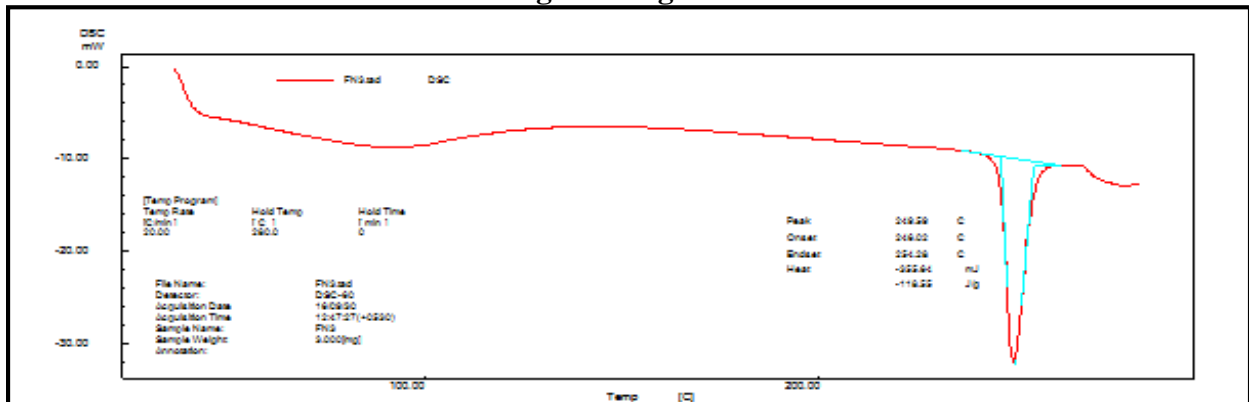


Figure No.2: DSC thermo grams of pure Nevirapine and Nevirapine loaded Eudragit L100 nanoparticles

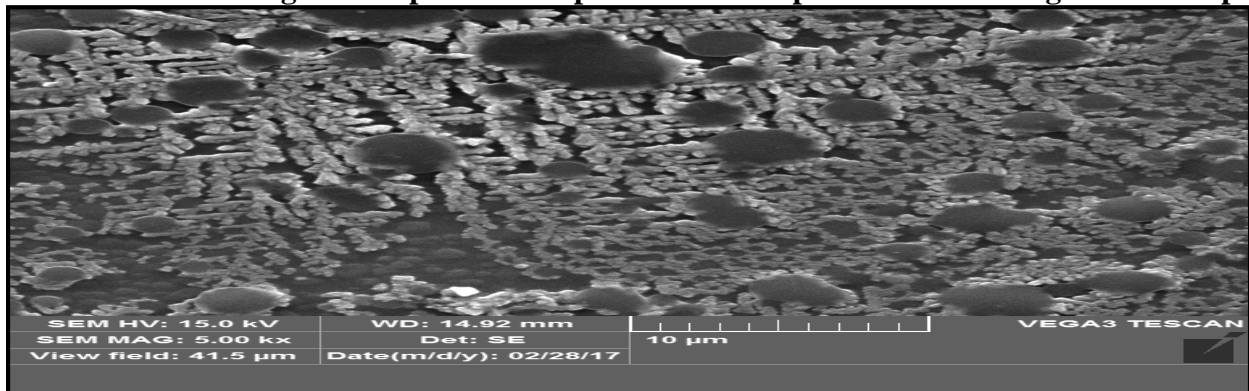


Figure No.3: SEM of formulation FE-4

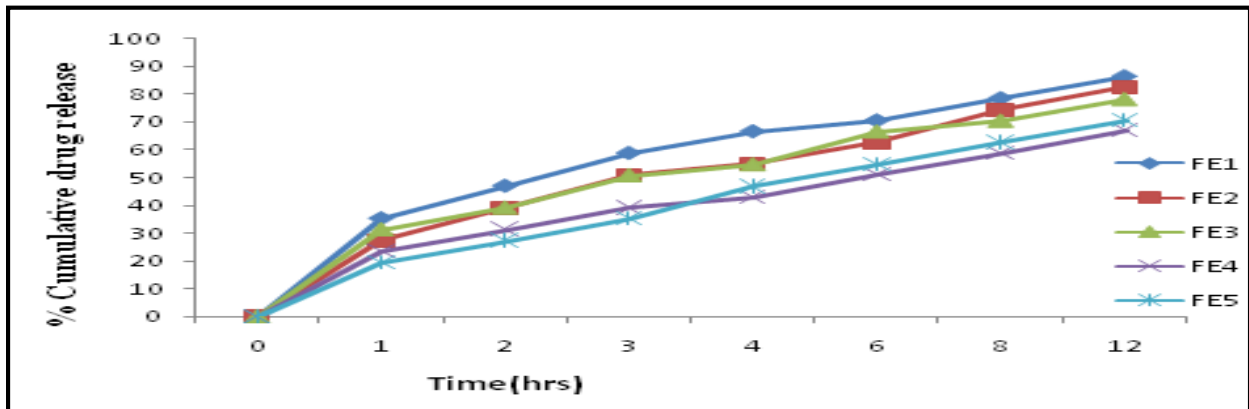


Figure No.4: % Cumulative drug release of Nevirapine nanoparticles

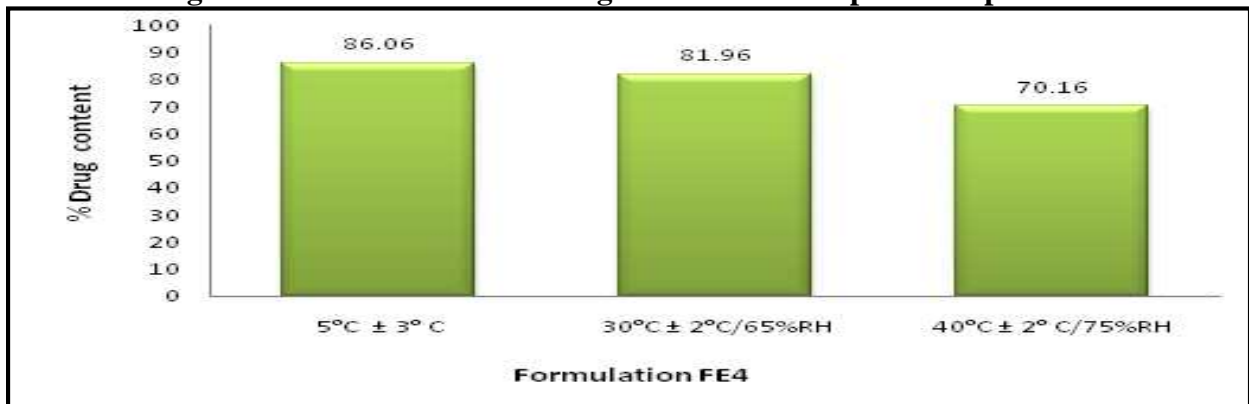


Figure No.5: Stability study: comparison of % drug content of formulation FE-5 at 5±3°, room temperature and 45° ± 2°/75% RH

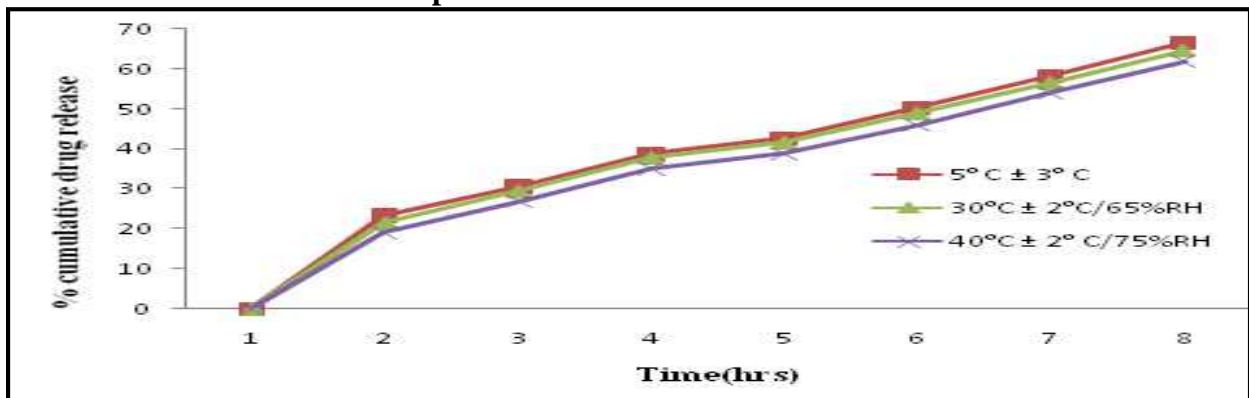


Figure No.6: Stability study: comparison of *in vitro* drug release profile for formulation FE-5 at 5±3°, room temperature and 45° ± 2°/75% RH after three months storage

## CONCLUSION

Nevirapine nanoparticles were prepared by Solvent evaporation technique were found to be suitable for controlled release. The nanoparticles prepared by using Eudragit L 100 as a polymer show prolonged release rate when compared with other formulations.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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